



Acute and Subacute Toxicity Studies of the Methanol Extract from Leaves of *Pisonia Aculeata* LINN.

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ABSTRACT

The methanol extract from the leaves of *Pisonia aculeata* Linn. (MPA) was evaluated for acute and subacute toxicity. The purpose of acute toxicity studies is to determine the LD₅₀ values which help in determining the safe dose range at which the drug can be used such that there is no harmful or lethal effect on the animal. No mortalities had occurred during acute toxicity studies show the MPA did not produce death even at high dose (2 g/kg) and so it is found to be safe. In subacute toxicity study, the MPA was administered orally at a dose of 100, 250 and 750 mg/kg for 28 days. It was observed that the animals fed with MPA were healthy. Haematological parameters found to be within the normal range. Biochemical parameters were not significantly different between the control and the experimental groups of rats. Analysis of the urinary metabolite levels showed trace or no presence.

Keywords: Acute toxicity, Sub acute toxicity, *Pisonia aculeata* Linn.

INTRODUCTION

Pisonia aculeata Linn. family, Nyctaginaceae^{1, 2} is a well-known medicinal plant that is frequently prescribed in various indigenous systems of medicines especially those of India, Tibet, China and Korea. It's most widespread traditional uses have been for the treatment of scabies, counterirritant for swellings and rheumatic pains.³ Hepatoprotective and Antioxidant Study⁴ showed a remarkable hepatoprotective and antioxidant properties of *P. aculeata* against CCl₄-induced hepatotoxicity in rats. Anti-Tumor Study on the ethanol extract of *P. aculeata* leaves on Ehrlich Ascites Carcinoma in mice⁵, Antitumor Activity of Methanolic Extract of *Pisonia Aculeata* Leaf⁶ showed a remarkable results. The findings of this study indicate that the MPA possesses significant antitumor activity on dose dependent manner⁷. The extract of *Pisonia aculeata* showed the positive test for alkaloids, glycosides, triterpenes, flavoind and phenolic compounds⁵. It chiefly consists of Cynogenic glycoside which is responsible for its different therapeutic activities⁸. Recently, the methanol extract from the leaves of *Pisonia aculeata* Linn. possess antitumor properties⁶ However, the toxicity of *Pisonia aculeata* Linn. has not been intensively studied. The methanol extract isolated from leaves, given separately in various doses (50, 500, 1000, 2000 mg/kg) by orally to Adult female Wistar rats (125-150 g), was shown to have the LD₅₀ 2g/kg. Our study was aimed to evaluate the oral acute and subacute toxicity of MPA in rats with the hope that the results would provide information on the safety of this extract prior to the evaluation of its therapeutic efficacy in humans.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Pisonia aculeata* Linn. were collected from the surrounding of Tirupati district (517502), Andhra Pradesh, India, in the month of April and May 2010 and authenticated by Dr. K. Madhava Chetty, Asst. Professor, Department of Botany, Sri Venkateshwara University, Tirupati, and Andhra Pradesh, India.

Preparation of plant extract

The leaves were shade dried and pulverized. The powder was treated with petroleum ether for de waxing and removal of chlorophyll. Later it was packed (250 g) in Soxhlet apparatus and subjected to continuous hot percolation for 8 h using 450 ml methanol (70% v/v) as solvent. The methanolic extract was concentrated under vacuum and dried in desiccators (yield 12.5 g, 5% w/w). (Abbreviated as **MPA**)

Experimental animals

Adult female Wistar rats (125-150 g) were used for acute and subacute toxicity. These animals were kept in polypropylene cages under identical animal house condition and provided with standard pellet and water ad libitum. Each cage contained 3 and 6 rats of the same sex with a bedding of husk, and 12-hour light/dark cycles were provided resp. Environmental conditions were maintained at a temperature of 22°C ± 2°C and a relative humidity of 60% ± 10%.⁹

Acute toxicity

According to the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals, 420.¹⁰ The overnight fasted rats were divided into 4 groups, each group consisting of 3 animals. The

methanol extract of whole plant of **MPA** was given separately in various doses (50, 500, 1000, 2000 mg/kg) by oral route. After administration of the extract, the animals were observed continuously for the first two hours and 24 hrs to detect changes in the behavioural responses and also for tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma and monitored for any mortality.

Subacute toxicity

According to OECD TG407¹¹, Four groups of rats received **MPA** at 100, 250 and 750 mg/kg (low dose, intermediate dose and high dose) orally for 28 days. The group which served as control received equivalent quantity of normal saline orally. Animals were observed for signs and symptoms, behaviour alteration, food and water intake and body weight changes. All animals were observed twice daily for mortality during the 28 day period of study. The weight of each rat was recorded on day 0 and at weekly intervals throughout the course of the study. The group means body weights were calculated.

At the end of the 28-day period the animals were fasted overnight. The following morning, each animal was heparinized and blood samples were collected from the orbital sinus. The blood sample was collected after 24 h of the last doses of alcoholic extracts.

Hematological analyses were performed in total blood was collected in heparinized funnels and tubes with EDTA. The relative blood indices white blood cell count (WBC), red blood cell count (RBC), haemoglobin (Hb), erythrocyte sedimentation rate (ESR), platelets, clotting time and packed cellular volume (PCV) were determined using routine method.⁸

In addition, Biochemical analyses were performed in serum obtained after centrifugation of total blood without anticoagulants, at 2400 rpm for 15 min. The analysis of blood glucose¹² Cholesterol¹³, Serum glutamate pyruvate (GPT)¹⁴ and glutamate oxaloacetate transaminase (GOT)¹⁴, Alkaline phosphatase, Total bilirubin¹⁵, Urea and BUN¹⁶, Total Protein & Albumin¹⁷ and creatinine¹⁸ were estimated in serum. Urine samples were also collected at the end of the study period and analyzed for specific gravity, pH, glucose, proteins, ketones, and occult blood. At the end of 28 days, experiment animals were autopsied and vital organs viz. Liver, kidney, spleen, lung, heart and brain were

removed and subjected to gross examination and later weighed.

Since liver and kidney are organs of metabolism and excretion, potentially toxic agents are likely to affect them. So, portions of these organs were fixed in buffered 10% formalin and 5 µm thick paraffin sections were made and stained with haemotoxylin and eosin¹⁹ for microscopic examination of rats.

Statistical analysis

The results were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Turkey multiple comparison tests. P values < 0.05 were considered as significant.

RESULTS AND DISCUSSION

It was observed that the animals fed with extract of **MPA** were healthy. No unusual changes in behavior or in locomotors activity, no ataxia, and no signs of intoxication were observed during the 28-day period. No differences were found in growth between the control group and the animals fed with different levels of the extract of **MPA**, (Table 1). No change in fur coating, eyes and respiratory functions. There was no significant difference in the food and water consumption between the treatment and control groups.

Table 1: Body weights of rats in subacute toxicity of MPA

Treatment	Dose (mg/kg)	Body weight (gm)	
		Initial	After 28 days treatment
Methanol extract of <i>P.aculeata</i>	-	146 ± 1.2	158 ± 1.6
	100	143 ± 1.4	160 ± 1.8
	400	148 ± 0.7	164 ± 2.1
	750	140 ± 1.1	162 ± 1.3

Values are expressed as mean ± SEM, n = 6, NS = Statistically not significant.

Haematological parameters, RBC, WBC (Total), Hb, ESR and PCV, in both control and experimental rats, were not significantly different (P >.05) for **MPA** (Table 2). But platelets and clotting time shows significant differences by statistically (P<0.01; P<0.05), the values were proximately closed to control group. All values were found to be within the normal range for rats, and there were no differences between the groups.

Table 2: Hematological values of rats in subacute toxicity of MPA

Treatment	Dose (mg/kg)	Hb gm %	RBC 10 ⁶ /cumm	Total WBC 10 ³ /cu.mm	ESR mm/1 st hr	Platelets (K/µL)	Clotting time (Sec)
Control	-	13.18 ± 0.56	4.76 ± 0.46	8.10 ± 0.24	3.05 ± 0.16	510 ± 17.18	130.5 ± 1.49
Methanol extract of <i>P.aculeata</i>	100	12.17 ± 0.35	4.56 ± 0.25	8.94 ± 0.17	3.12 ± 0.18	526 ± 15.24	127.83 ± 1.42
	400	13.82 ± 0.52	4.06 ± 0.38	8.46 ± 0.26	3.40 ± 0.10	605 ± 21.58 ^a	124.13 ± 1.78 ^b
	750	14.23 ± 0.54	4.69 ± 0.65	7.93 ± 0.14	3.52 ± 0.28	598 ± 19.25 ^b	125.16 ± 1.10

Values are expressed as mean ± SEM, n = 6, ^aP<0.01; ^bP<0.05 Vs control



Table 3: Blood chemistry values of rats in sub acute toxicity of the MPA

Treatment	Dose (mg/kg)	Blood Glucose mg%	Cholesterol mg%	SGPT U/L	SGOT U/L	ALP U/L	Total Bil mg%
Control	-	96 ± 1.7	126 ± 1.70	110 ± 1.9	47.5 ± 1.3	190 ± 2.10	0.80 ± 0.04
Methanol extract of P.aculeata	100	102 ± 2.0	110 ± 1.30	112.6±2.7	52.0 ± 1.7	198 ± 2.36	0.79 ± 0.05
	400	98 ± 1.6	118 ± 1.95	118.5±2.6	49.0 ± 2.4	188 ± 2.50	0.86 ± 0.02
	750	90 ± 2.14	123 ± 2.86	121.0±2.1	51 ± 2.0	192 ± 2.0	0.78 ± 0.06

Values are expressed as mean ± SEM., n=6, NS=statistically not significant

Table 4: Blood chemistry values of rats in subacute toxicity of MPA

Treatment	Dose (mg/kg)	Urea mg %	BUN mg%	Creatinine mg %	Total Protein gm%	Albumin gm%
Control	-	36.5 ± 1.14	28.65 ± 2.13	0.9 ± 0.10	8.3 ± 0.96	4.07 ± 0.54
Methanol extract of P.aculeata	100	38.7 ± 1.43	25.79 ± 1.17	0.8 ± 0.02	7.9 ± 0.65	4.13 ± 0.29
	400	37.5 ± 1.57	27.24 ± 1.78	0.9 ± 0.07	8.5 ± 0.72	4.27 ± 0.24
	750	40.2 ± 2.0	30.63 ± 2.34	1.0 ± 0.09	7.8 ± 0.49	4.33 ± 0.73

N=6 animal in EPACH group; Values are expressed as mEPAN ± SEM; NS = Statistically not significant

Table 5: Urine analytical values of rats in subacute toxicity of MPA

Treatment	Dose (mg/kg)	Specific gravity	pH	Glucose	Protein	Ketone bodies	Blood cells
Control	-	1.02 ± 0.04	6.72 ± 0.19	NIL	NIL	NIL	NIL
Methanol extract of P.aculeata	100	1.03 ± 0.06	6.58 ± 0.18	NIL	NIL	NIL	NIL
	400	1.04 ± 0.03	6.09 ± 0.16	NIL	NIL	NIL	NIL
	750	1.06 ± 0.02	6.25 ± 0.24	NIL	NIL	NIL	NIL

N=6; Values were expressed as Mean ± SEM, NS- Not significant

Table 6: Organ weights of rats in subacute toxicity of MPA

Treatment	Dose (mg/ kg)	Liver	Kidney	HEPArT	Lungs	Spleen	Brain
Control	-	3.46 ± 0.12	1.17 ± 0.19	0.68 ± 0.03	1.25 ± 0.14	0.95 ± 0.07	1.65 ± 0.58
Methanol extract of P.aculeata	100	3.75 ± 0.10	1.13 ± 0.17	0.76 ± 0.04	1.15 ± 0.14	0.90 ± 0.08	1.59 ± 0.36
	400	3.52 ± 0.21	1.27 ± 0.23	0.69 ± 0.02	1.28 ± 0.13	1.06 ± 0.06	1.69 ± 0.24
	750	3.70 ± 0.13	1.10 ± 0.20	0.70 ± 0.05	1.30 ± 0.16	1.15 ± 0.18	1.75 ± 0.38

Values are expressed as mean ± SEM, n=6, NS=statistically not significant

The levels of serum analytes, such as glucose, Cholesterol, Serum glutamate pyruvate (GPT) and glutamate oxaloacetate transaminase (GOT), Alkaline phosphatase, Total bilirubin, Urea, BUN, Total Protein, Albumin and creatinine were not significantly different between the control and the experimental groups of rats ($P > .05$) when fed with **MPA** (Tables 3 and 4). Analysis of the urinary metabolite levels (specific gravity, pH, glucose, proteins, blood cells, and ketones) showed trace or no presence of these in both control and experimental animals fed with extracts (Table 5).

The organ-to-body mass ratios of animals at the end of 28 days' feeding. No abnormal changes were observed in organ mass with respect to body mass of extract of **MPA** fed rats in comparison with controls. Observations of gross pathology immediately after dissection, on rats of all groups were found to be uniformly healthy, lacking in any apparent pathological abnormalities (Table 6).

Histopathological examination of the liver and kidneys in the control and the **MPA** extract fed groups showed no differences, indicating that feeding these **MPA** extract of whole plant at these levels to the rats did not result in any adverse toxicological effect on these organs.

The purpose of this study was to look at the toxicity profile of the **MPA**. A 28-day study is considered a sub acute study, which is well accepted for eliciting any toxicity on long-term feeding. It gives valuable information on the cumulative toxicity of a substance on target organs or physiological and metabolic effects of the compound at low dose on prolonged exposure. A wide variety of adverse effects can be detected from sub acute toxicity studies. The result from such studies can provide information, which will aid in selecting dose level. The long term safety level of a compound can be predicted from acute or shorter than sub acute studies. Acutely nontoxic compounds may be toxic on prolonged

exposure even at low dose levels due to accumulation, changes in enzyme level and disruption of physiological and biochemical homeostasis. Sub acute studies are generally carried out in few days to three months.

According to Onyenyili and co-workers²⁰, anemia following administration of an agent can be as a result of lysis of blood cells and/or inhibition of blood cell synthesis by the active constituents of the extract, and decrease in hematological parameters in experimental animals has been associated with anemia²¹. There was no significant change in hematological parameters in the extract-treated animals compared to the control (Table 2), which indicates that there is no lysis of blood cells and/or inhibition in blood cells synthesis by the active constituents of **MPA** extract. The above results suggest the non toxicity of **MPA** in rats. Clinical chemistry parameters were statistically not significant in comparison to control groups. They are confirming the absence of adverse effects. No adverse effect has been observed in renal function test also. No mortality was recorded in rats treated with higher dose of 700 mg/kg. The toxicity study of the drug reflected the innocuous nature of this plant extracts on hepatic, renal and haemopoetic system even at high dose.

Rats treated with the various doses of the extract (200, 400 and 700 mg/kg) had no significant change in body weight. No statistically significant differences existed in the absolute and relative weights of all the isolated organs between the treated and the control rats (Table 6). Kluwe¹² documented that the absolute organ weight has been observed to be a relative sensitive indicator of nephrotoxicity for known nephrotoxicants. An increase in kidney weight (either absolute or relative) indicates nephrotoxicity²². The **MPA** did not induce any toxic effect on the kidneys and the other organs going by this indicator, since the absolute and relative weights of the organs were not significantly different from control values.

Microscopic data together with the data of macroscopic evaluation of the animals' organ showed that both test and control groups were practically healthy. According to the data of histological examination, no toxic or allergic effects of **MPA** were detected in the test group. No local irritating effects of the drug preparation were observed in the test group during the study period. Our experiments showed that **MPA** has a safe.

In histopathological studies, the liver of treated animals showed normal histological feature at 200, 400 and 700 mg/kg. No degeneration of hepatocyte, focal steatosis, congestion of central vein and inflammation of portal tract when compared with control animals. The kidney of treated rats showed normal glomeruli and there is no necrosis of tubular epithelium in the kidney. Gross examination of liver and kidney on histology did not reveal any abnormalities. Thus, it was concluded that **MPA** did not produce any toxic effect in male albino rats.

No mortality was recorded in experimental animals treated with the drug orally at a dose of 700 mg/kg. The toxicity study of the drug reflected the innocuous nature of this plant extracts on hepatic, renal and haemopoetic system even at high dose level of daily administration for 28 days in rats.

CONCLUSION

Methanolic Extract of *Pisonia aculeata* leaf Linn. (**MPA**) can be considered safe, as it did not cause either any lethality or adverse changes with general behaviour of rats in acute toxicity study up to the dose of 2000 mg/kg b.wt. and also there were no observable detrimental effects caused by **MPA** (up to 700 mg/kg b.wt.) in sub acute toxicity study in rat model. Further, the above results sub-ststantiate the beneficial and enhanced pharmacological effects with the independent activity of **MPA**.

In summary, the **MPA** was found to be fairly nontoxic when oral acute and subacute toxicities in rats were performed.

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